DISTYLY AND MONOMORPHISM IN *VILLARSIA* (MENYANTHACEAE): SOME EVOLUTIONARY CONSIDERATIONS[1]

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ABSTRACT

Distyly occurs in four of the five genera of the Menyanthaceae, as do breeding systems such as dioecy, gynodioecy, and homostyly that are believed to be derived from distyly. Most species of the largely Australian genus Villarsia *are distylous, but the Western Australian V. albiflora has monomorphic (nonheterostylous) flowers and appears to be homostylous. Despite this, individuals of this species are self-incompatible, although most members of the four populations studied are intercompatible. The incompatibility system of V. albiflora appears to be controlled by multiple alleles. Another Western Australian species, V. parnassiifolia, has distylous flowers and strong self-incompatibility. Long × Long crosses of this species fail to produce seeds, but Short × Short crosses commonly produce a full complement of seeds. Thus, this species possesses a self-incompatibility system, but Shorts have an unexpectedly high degree of intramorph intercompatibility, suggesting that the incompatibility system of this morph likewise is controlled by multiple alleles. Five of eight natural populations sampled of V. parnassiifolia showed Short-dominated morph ratios, suggesting that offspring of Short × Short pollinations may constitute a portion of these Short-dominated natural populations.* Villarsia albiflora *may represent a recombinant homostyle, a true-breeding Short morph derived from a distylous ancestor with a breeding system similar to that of V. parnassiifolia, or a species possessing a floral morphology and breeding system ancestral to the distyly that occurs widely in the Menyanthaceae. A scheme for the origin of distyly in* Villarsia *is presented.*

The Menyanthaceae consist of five genera of wetland or aquatic herbs. *Menyanthes* and *Fauria* (*Nephrophyllidium*) are monotypic north-temperate genera, both of which bear distylous flowers. *Nymphoides* is widely distributed in tropical and temperate regions of both hemispheres; distyly is common in this large genus, as are derivative breeding systems such as dioecy, gynodioecy, and autogamy associated with homostyly (Ornduff, 1966, 1970b, 1973; Vasudevan Nair, 1975). *Liparophyllum* is a monotypic genus restricted to New Zealand and Tasmania; its flowers are monomorphic and the species is self-compatible (Ornduff, 1973). *Villarsia* occurs in southeastern Asia, South Africa, and Australia, with the largest concentration of species in southwestern Western Australia. Several Australian *Villarsia* species have distylous flowers and an associated self- and intramorph incompatibility system (Ornduff, 1974, 1982, 1986, 1988; Fig. 1). My recent work has revealed that some species of *Villarsia* with distylous flowers have various altered incompatibility systems; one

of these species, the Western Australian *V. parnassiifolia* (Labill.) R. Brown, is discussed in this paper. Another species, *V. albiflora* F. Muell., is a Western Australian species which has nonheterostylous flowers (Fig. 1), a self-incompatibility system, and essentially full intercompatibility among members of a population. This species is thus not the self-compatible homostyle of the type that commonly occurs in various other predominantly distylous genera (Richards, 1986). This paper documents the nature of the incompatibility systems of *V. parnassiifolia* and *V. albiflora* and discusses the possible relationships between the unique breeding system of *V. albiflora* and those that occur in other species of *Villarsia*. Since work on these two species, as well as others of the genus, is still in progress, this report is preliminary.

MATERIALS AND METHODS

Seeds were collected in 1983 from four populations of *Villarsia albiflora* in Western Australia:

Long-styled   Short-styled

Villarsia parnassiifolia (9404)

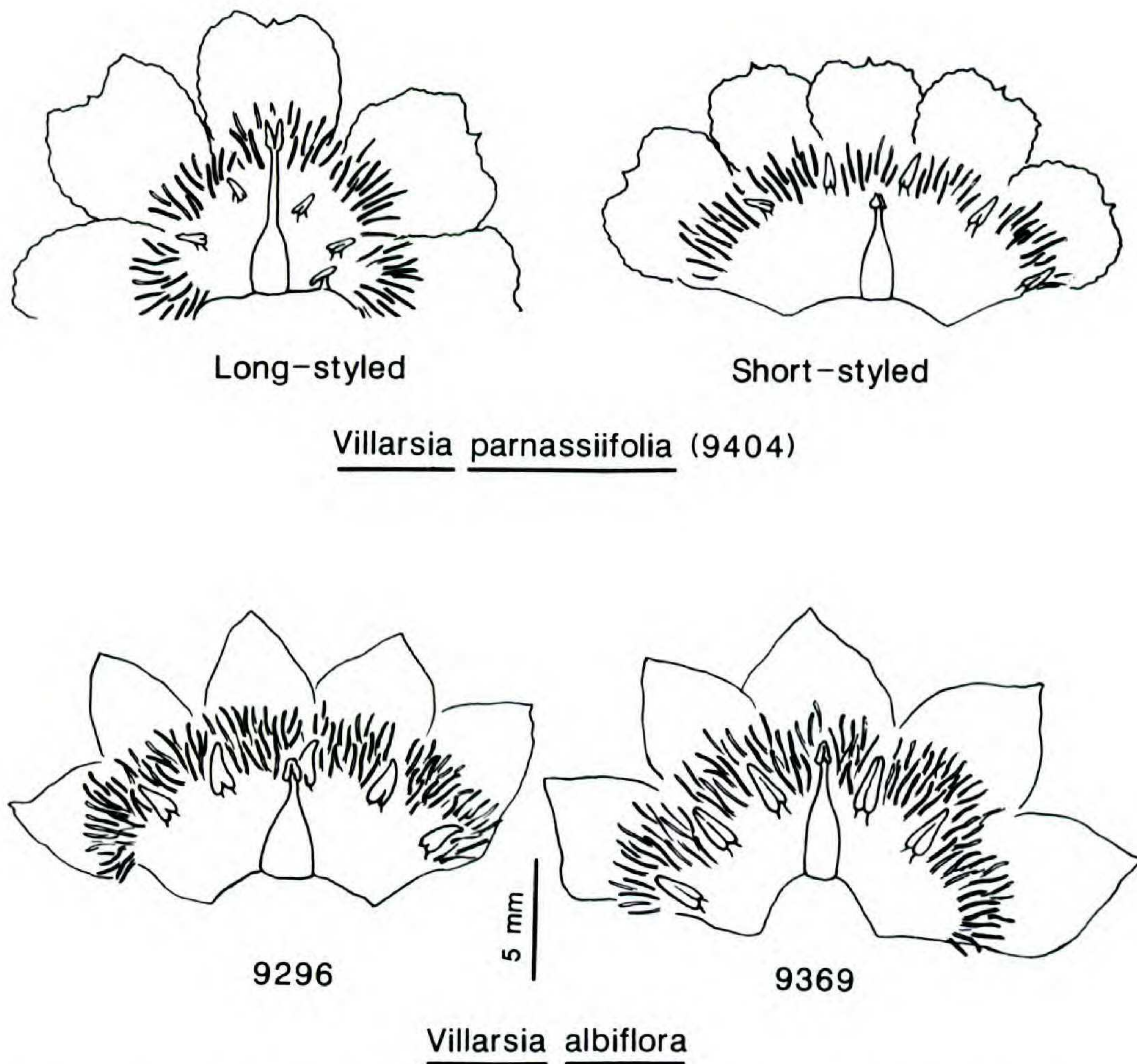9296   5 mm   9369

Villarsia albiflora

FIGURE 1.   *Dissections of distylous flowers of* Villarsia parnassiifolia *and of monomorphic flowers of* V. albiflora.

9296, Gnangara Lake, near Perth; 9365, Medina, near Perth; 9369, the Capel-Boyanup area; and 9397, near Mount Chudalup. This sampling encompasses most of the geographical range of this species, which is diploid (Ornduff & Chuang, in press). At the same time, seeds were also collected from two populations of *V. parnassiifolia*: 9404, a diploid population near Walpole, and 9413, a tetraploid population at Parry Beach, near Denmark (Ornduff & Chuang, in press), both occupying central positions in the range of this species along the southern coast of Western Australia. Plants were grown from these seeds in the greenhouses at the University of California, Berkeley. Thirty-eight individuals of *V. albiflora* and 10 long-styled plants (Longs) and 13 short-styled individuals (Shorts) of *V. parnassiifolia* were used in the crossing program. Each individual in a progeny was assigned a plant number for reference purposes. Each plant was self-pollinated and crossed with as many other individuals in the population as possible during the spring and summer of 1986 and 1987. At least six pollinations for each type of cross were performed. Nearly mature capsules were collected individually in seed envelopes, and the number of seeds counted. Flowers of self- and cross-pollinated plants of both species were collected 24 hours after pollination, the gynoecium excised, mounted, stained, and observed under ultraviolet light to observe the behavior of pollen and pollen tubes following various types of pollinations (using the method of Martin, 1959). Seeds of intra- and intermorph crosses of *V. parnassiifolia* (population 9413) were sown in a greenhouse, the seedlings grown to flowering, and the style lengths of each individual recorded.

RESULTS

All seed-set figures were assigned arbitrarily to one of three categories (Figs. 2, 3). "High" seed production refers to crosses in which all pollinations produced capsules with large numbers of seeds; "intermediate or variable" seed production includes crosses in which seed production was markedly lower than the "high" category of that seed parent, or in which some crosses failed to produce seed; and "low" seed production refers to crosses producing few or no seeds. These categories will not be quantified in this paper because of high variances in the first two categories and the small number of pollinations (a minimum of six) con-
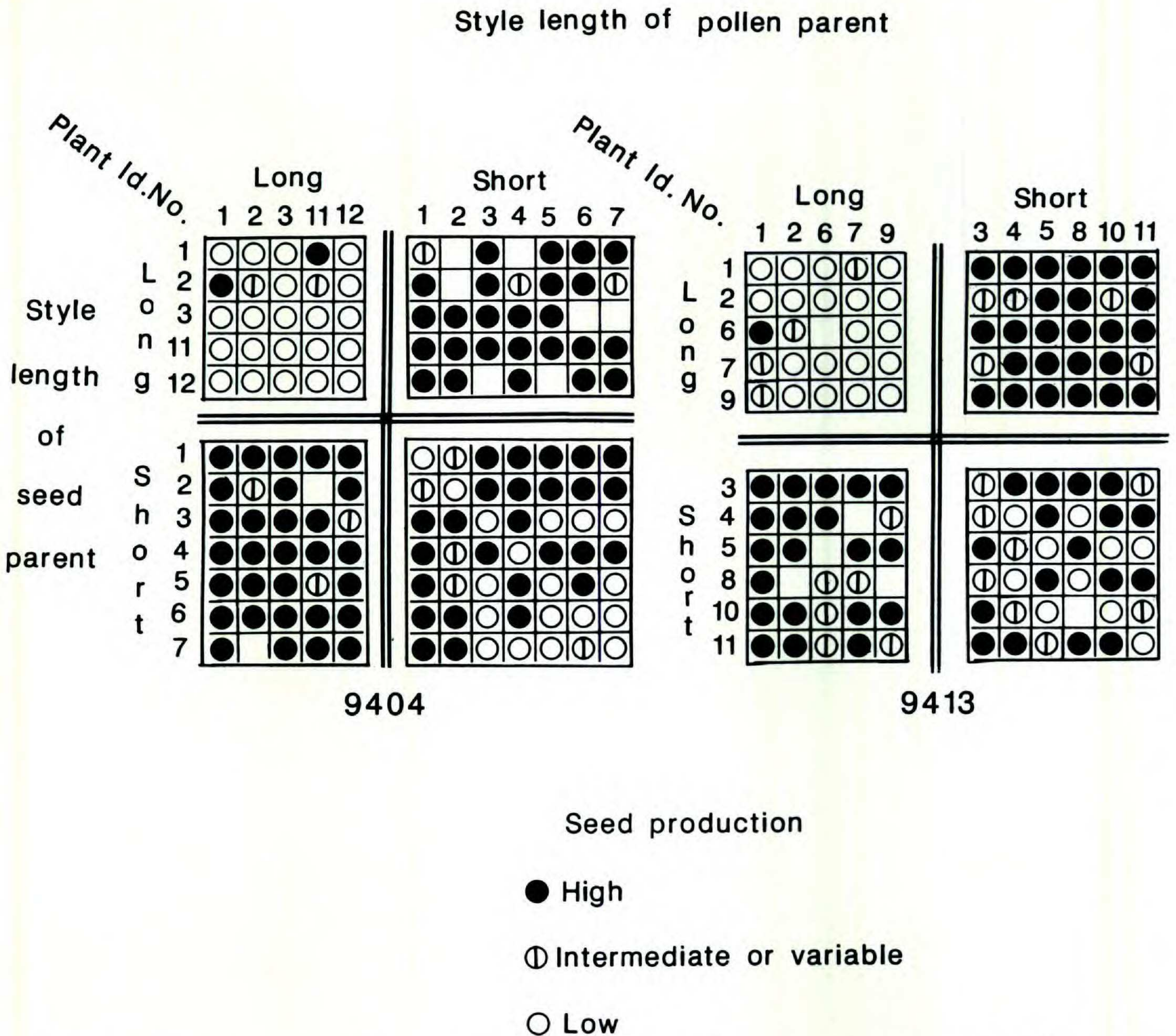
FIGURE 2. *Seed production following artificial self- and cross-pollinations of the two morphs of the distylous* Villarsia parnassiifolia, *using two greenhouse-grown populations. The vertical and horizontal series of numbers refer to individual plants in each population. Seed production figures are described in text.*

ducted for each cross. Additional work is planned to increase the sample sizes. However, I am confident that the "high" and "low" categories represent two distinct types of results and provide a meaningful and relatively consistent basis on which to discuss the nature of the breeding systems in the two *Villarsia* species discussed in this paper. Whether the "intermediate or variable" category represents something other than a procedural artifact awaits additional data.

Villarsia parnassiifolia. Longs and Shorts of this species are strongly self-incompatible; only one of the nine selfed Longs and one of the 13 selfed Shorts produced any seeds following selfing (Fig. 2). Intermorph pollinations produced generally high seed-sets: 50 of the 58 Long × Short pollinations and 51 of the 60 Short × Long pollinations produced high seed-sets. Only three of the 40 Long ×

Long crosses produced a high seed-set, and an additional six of these crosses produced some seeds, indicating a high level of intramorph incompatibility of Longs. In contrast, 43 of the 71 Short × Short crosses produced high seed-sets, and an additional 13 of the crosses produced some seed, indicating a high level of intramorph compatibility of Shorts. Each Short in the two populations was fully compatible with at least one other Short in that population, and a few Shorts were compatible with most other Shorts in the same population. Most successful Short × Short crosses were successful in both directions, and most that failed did so in both directions. No differences were noted in behavior between the diploid and the tetraploid populations of *V. parnassiifolia.*

Crosses between four Longs and four Shorts of tetraploid population 9413 all produced both Longs
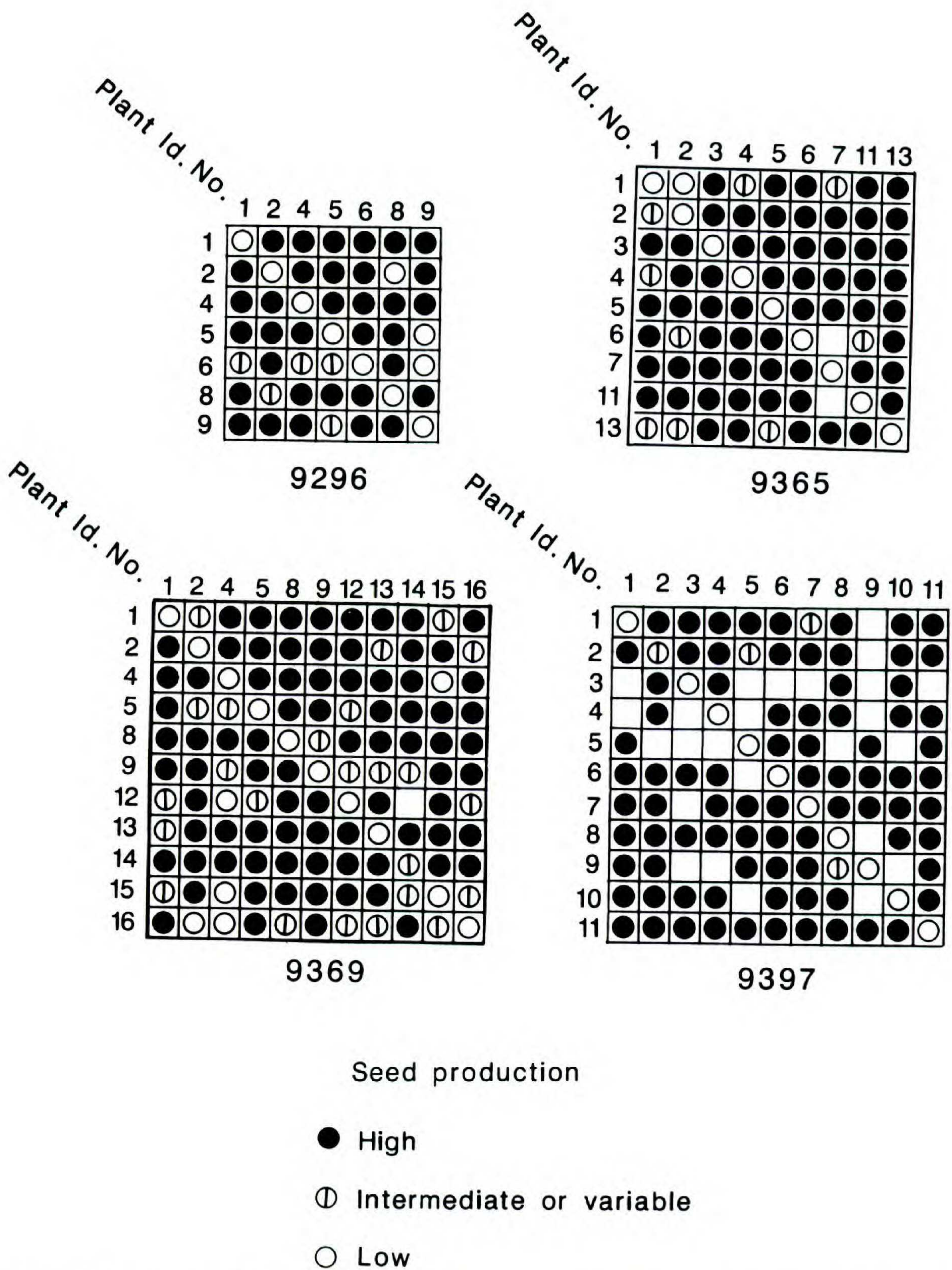
FIGURE 3. *Seed production following artificial self- and cross-pollinations of the monomorphic* Villarsia albiflora, *using four greenhouse-grown populations. The vertical and horizontal series of numbers refer to individual plants in each population.*

and Shorts in each progeny. Five Shorts of this population used in five Short × Short crosses produced Longs only in one progeny, Shorts only in two progenies, and Longs and Shorts in two progenies. Two 9413 Shorts selfed produced Longs and Shorts in each progeny. Two Long × Long crosses produced only Longs in their progenies. Although the progeny sizes are small, these results make it likely that the Shorts carry a dominant allele and Longs are homozygous recessive.

Villarsia albiflora. Results of pollinations using 38 individuals in four populations of *V. albiflora* provided relatively consistent results. All individuals proved to be self-incompatible, producing few or no seeds upon self-pollination (Fig. 3). Pollen grains on selfed stigmas either failed to germinate or germinated with growth of the pollen tube into stigmatic tissue but not further. Most individuals produced high seed-sets when crossed with other individuals in the population. Most crosses that

failed in one direction were successful in the other direction, or provided seed-sets in the "intermediate or variable" category in that direction. Only one instance was found of apparent bilateral incompatibility (between plants 4 and 16 of population 9369). Thus, the nonheterostylous *Villarsia albiflora* has a pronounced self-incompatibility system, but most members of each population are intercompatible.

DISCUSSION

Villarsia parnassiifolia has morphologically distylous flowers and a strong self-incompatibility system. Although Long × Long crosses generally failed to produce seeds, most Short × Short crosses were fully compatible, producing high seed-sets. No differences were observed in the behavior of diploids and tetraploids. Self- and intramorph incompatibility are commonly associated with distyly, and in most examples where intramorph compatibility exists, as in some species of *Hedyotis* (Rubiaceae), *Melochia* (Sterculiaceae), and *Amsinckia* (Boraginaceae), it is associated with self-compatibility as well (Ganders, 1979).

The occurrence of self-incompatibility but intramorph compatibility in distylous species is rare. It has been reported in the borages *Anchusa hybrida* Ten. (Dulberger, 1970) and *A. officinalis* L. (Philipp & Schou, 1981). I am reluctant to consider the examples of *Narcissus tazetta* L. and *Mirabilis froebelii* (Behr) Greene cited by these authors to represent heterostyly. In *Anchusa officinalis*, Schou & Philipp (1984) demonstrated that the morphological features of distyly are controlled by a single diallelic locus, with Longs homozygous recessive and Shorts with one or two dominant alleles, which is the common genetic basis of distyly (Ganders, 1979). However, as Dulberger (1970) suggested is the case for *Anchusa hybrida*, the incompatibility system of *A. officinalis* is controlled by at least two alleles, and these segregate independently from those controlling the morphological features of distyly (Schou & Philipp, 1984).

The condition described in *Villarsia parnassiifolia* resembles that of the two *Anchusa* species but differs in that the Long but not the Short morph of *V. parnassiifolia* possesses intramorph- as well as self-incompatibility. It would appear that in the Long morph of this species the incompatibility alleles are linked to the "morphological" locus, but in the Short morph they are not. How this is accomplished (if it is) is unclear.

Style lengths of the few small progenies obtained by self- and intramorph pollinations of the tetraploid population of *Villarsia parnassiifolia* are

consistent with the notion that the Short morph of this species carries a dominant allele with Longs thus homozygous recessive, but exact interpretation of the scant data may be obscured by tetrasomic inheritance. Short-dominated morph ratios occur in five of the eight field populations sampled of this species (Ornduff, 1986; $P = <0.05$ with the Wilcoxon's signed-ranks test), suggesting that under field conditions Short × Short crosses commonly participate in contributing to the composition of natural populations.

The floral morphology of *Villarsia albiflora* suggests that it is a homostyle, but this tentative conclusion requires examination. In genera or families with both distyly and homostyly, the homostylous condition is usually viewed as the result of genetic recombination in the S "supergene," leading to production of flowers combining carpel characters of one morph with stamen characters of the other morph. Commonly, such homostyles are Long-homostyles, although Short-homostyles are also known. Homostyly has been recorded in diverse genera such as *Armeria* (Plumbaginaceae; Baker, 1966), *Gelsemium* (Loganiaceae; Ornduff, 1970c), *Limonium* (Plumbaginaceae; Baker, 1953), *Nymphoides* (Menyanthaceae; Ornduff, 1970a), *Oldenlandia* (Rubiaceae; Bir Bahadur, 1970), *Piriqueta* (Turneraceae; Ornduff, 1970a), *Primula* (Primulaceae; Darwin, 1877; Ernst, 1955), *Turnera* (Turneraceae; Urban, 1883; Barrett & Shore, 1987), *Villarsia* (Menyanthaceae; Ornduff, 1974), and *Waltheria* (Sterculiaceae, Bir Bahadur, 1977). Because a single homostyle flower usually bears a combination of pollen of one morph with carpels of the other morph, such homostyles are generally self-compatible and sometimes largely autogamous. Other types of homostyles occur in *Amsinckia* (Boraginaceae; Ray & Chisaki, 1957), *Hedyotis caerulea* (Rubiaceae; Ornduff, 1977), *Mitchella repens* (Rubiaceae; Ganders, 1975), and *Primula* (Primulaceae; Ernst, 1955). The nature and placement of anthers and stigmas of these homostyles vary, but cannot be attributed to genetic recombination alone and must involve the additional action of modifier genes. Such homostyles may be self-compatible (as in *Amsinckia* and some species of *Primula*) or self-incompatible (as in *Mitchella repens*, *Hedyotis caerulea*, and some species of *Primula*). In the latter examples, self-incompatible homostyles appear to be very rare or known only from cultivated material (Ganders, 1975). Once homostyly has developed, even as a result of simple genetic recombination, carpel, stamen, and other floral traits may subsequently be altered by modifier genes to accommodate the homostyly in the direction of greater autogamy (as apparently is the case

in *Piriqueta cistoides*, Ornduff, 1970a) or in the direction of greater xenogamy (as apparently is the case for some races of *Turnera ulmifolia*; Barrett, 1988).

The nature and origin of the floral monomorphism of *Villarsia albiflora* are not clear. If we assume for purposes of discussion that this species represents a recombinant homostyle, its self-incompatibility and essentially full intercompatibility are consistent with the idea that style-length and incompatibility reactions are not uniformly controlled by sets of linked alleles in distylous *Villarsia* species. However, since incompatibility of Short pollen but not Long pollen appears to be unlinked to style length, this may require that the putative homostyly of *V. albiflora* is Long-homostyly (i.e., the style of Longs combined with anther position and incompatibility behavior of Shorts in one flower) rather than Short-homostyly. Whether this supposition will survive the scrutiny of further study remains to be seen.

A second, perhaps more attractive (or at least potentially less flawed) hypothesis is that the monomorphism of *V. albiflora* represents a situation in which the flowers of this species are fundamentally Shorts, and in which the Longs of a presumed distylous ancestor have been lost. The anther position and stylar morphology of *V. albiflora* resemble those of Shorts of *V. parnassiifolia* more closely than they do those of Longs of that species (Fig. 1). Short × Short pollinations of *V. parnassiifolia* are mostly compatible ones that produce vigorous offspring under artificial and, apparently, natural conditions. It is possible that in the evolution of *V. albiflora*, a postulated distylous ancestor lost the *s* allele and thus the Long morph, leading ultimately to a condition where populations consist of true-breeding homozygous Shorts carrying only the dominant *S* allele. If this postulated ancestor possessed the breeding system and Short-dominated morph ratios characteristic of *V. parnassiifolia* today, periodic severe reductions in population size as a consequence of the cyclic fluctuations in annual rainfall that have characterized southwestern Western Australia since mid or late Tertiary times (Hopper, 1979) might have resulted in the loss of the *s* allele and thus the loss of Longs. This would result in true-breeding homozygous Shorts as the exclusive components of surviving populations. Since *Villarsia* typically occurs in mesic to aquatic circumstances, recurrent xerothermic periods could have had strong effects on population sizes and distribution of species in this genus.

Because of the occurrence of distyly in four of the five genera of Menyanthaceae, it is natural to

assume that the floral condition and incompatibility system of *V. albiflora* are derivative ones from a distylous antecedent. A third evolutionary scenario contrary to this directionality in evolution is that the floral monomorphism of *V. albiflora* is primary in the genus and that distyly elsewhere in *Villarsia* has been derived from this type of monomorphism. Starting with a self-incompatible nonheterostylous species similar to *Villarsia albiflora* (which today shows some interpopulation variability in position of stigmas relative to anthers, Fig. 1; Ornduff, 1986), selection might operate to favor increased distance between stigmas and anthers as a means of reducing pollen wastage by selfing. One means of achieving this could be via style-length dimorphism associated with a slight shifting of anther position (the positions of anthers in the two morphs of the distylous *V. parnassiifolia* are not very different; Fig. 1). Initially, the alleles controlling floral dimorphism would be unlinked to those multiple alleles controlling the incompatibility reaction (as in *Anchusa*). Gradually, linkage between these two sets of alleles would develop with a concomitant decrease in the number of incompatibility alleles; in *Villarsia parnassiifolia* such a linkage appears to occur in Longs but not in Shorts. Ultimately, the alleles controlling floral dimorphism would be tightly linked to those controlling incompatibility, the latter having been reduced to a pair of alleles at a single locus. At this point, distyly of the conventional type that occurs in a variety of unrelated genera would have been achieved. In *Villarsia*, such "conventional" distyly and incompatibility occur in *V. capitata* Nees (Ornduff, 1982) and *V. congestiflora* F. Muell. (Ornduff, 1988).

Assuming this latter scheme represents an approximation of the sequence of events in the evolutionary history of *Villarsia*, it would explain an apparent anomaly that I commented on many years ago, namely that *V. capitata* and *V. congestiflora* "possess an unusual combination of highly advanced characters" with the "primitive" one of distyly (Ornduff, 1982). If distyly is indeed a condition that has developed from monomorphism within *Villarsia*, this anomaly is resolved, since distyly is thus viewed as advanced and not primitive.

This last suggested series of events is highly speculative. When more information becomes available on the breeding systems of other species of *Villarsia*, this third scenario should be evaluated in the context of different suggestions concerning the mode of origin of distyly proposed or discussed by Charlesworth & Charlesworth (1979), Ganders (1979), Muenchow (1982), and Gibbs (1986).

Clearly, breeding systems in the Menyanthaceae merit further experimental work and theoretical consideration.

LITERATURE CITED

BAKER, H. G. 1953. Dimorphism and monomorphism in the Plumbaginaceae. II. Pollen and stigmata in the genus *Limonium*. Ann. Bot. (London) 17: 433–445.

————. 1966. The evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. Evolution 20: 349–368.

BARRETT, S. C. H. 1988. The evolution, maintenance and loss of self-incompatibility systems. *In*: J. Lovett Doust & L. Lovett Doust (editors), Plant Reproductive Ecology: Patterns and Strategies. Oxford Univ. Press, New York.

———— & J. S. SHORE. 1987. Variation and evolution of breeding systems in the *Turnera ulmifolia* complex (Turneraceae). Evolution 41: 340–354.

BIR BAHADUR. 1970. Homostyly and heterostyly in *Oldenlandia umbellata* L. J. Genet. 60: 192–198.

————. 1977. Heterostyly, homostyly and incompatibility in *Waltheria indica* L. (Sterculiaceae). *In*: C. P. Malik (editor), Advances in Plant Reproductive Physiology. Kalyani Publishers, New Delhi.

CHARLESWORTH, D. & B. CHARLESWORTH. 1979. A model for the evolution of distyly. Amer. Naturalist 114: 467–498.

DARWIN, C. 1877. The Different Forms of Flowers on Plants of the Same Species. John Murray, London.

DULBERGER, R. 1970. Floral dimorphism in *Anchusa hybrida* Ten. Israel J. Bot. 19: 37–41.

ERNST, A. 1955. Self-fertility in monomorphic primulas. Genetica 27: 391–448.

GANDERS, F. R. 1975. Fecundity in distylous and self-incompatible homostylous plants of *Mitchella repens* (Rubiaceae). Evolution 29: 186–188.

————. 1979. The biology of heterostyly. New Zealand J. Bot. 17: 607–635.

GIBBS, P. E. 1986. Do homomorphic and heteromorphic self-incompatibility systems have the same sporophytic mechanism? Pl. Syst. Evolution 154: 285–323.

HOPPER, S. D. 1979. Biogeographical aspects of speciation in the southwest Australian flora. Ann. Rev. Ecol. Syst. 10: 399–422.

MARTIN, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol. 34: 125–128.

MUENCHOW, G. 1982. A loss-of-alleles model for the evolution of distyly. Heredity 49: 81–93.

ORNDUFF, R. 1966. The origin of dioecism from heterostyly in *Nymphoides* (Menyanthaceae). Evolution 20: 309–314.

————. 1970a. Relationships in the *Piriqueta caroliniana–P. cistoides* complex (Turneraceae). J. Arnold Arbor. 51: 492–498.

————. 1970b. Cytogeography of *Nymphoides* (Menyanthaceae). Taxon 19: 715–719.

————. 1970c. The systematics and breeding system of *Gelsemium* (Loganiaceae). J. Arnold Arbor. 51: 1–17.

————. 1973. Taxonomy. *In*: Menyanthaceae Dum., by S. Nilsson. World Pollen & Spore Fl. 2: 1–2, 11.

————. 1974. Cytotaxonomic observations in *Villarsia* (Menyanthaceae). Austral. J. Bot. 22: 513–516.

————. 1977. An unusual homostyle in *Hedyotis caerulea* (Rubiaceae). Pl. Syst. Evolution 127: 293–297.

————. 1982. Heterostyly and incompatibility in *Villarsia capitata* (Menyanthaceae). Taxon 31: 495–497.

————. 1986. Comparative fecundity and population composition of heterostylous and non-heterostylous species of *Villarsia* (Menyanthaceae) in Western Australia. Amer. J. Bot. 73: 282–286.

————. 1988. Distyly and incompatibility in *Villarsia congestiflora* (Menyanthaceae) with comparative remarks on *V. capitata*. Pl. Syst. Evolution 159: 81–83.

———— & T. I. CHUANG. 1988. Chromosome numbers of Western Australian species of *Villarsia* (Menyanthaceae). Pl. Syst. Evolution (in press).

PHILIPP, M. & O. SCHOU. 1981. An unusual heteromorphic incompatibility system. Distyly, self-incompatibility, pollen load, and fecundity in *Anchusa officinalis* (Boraginaceae). New Phytol. 89: 693–703.

RAY, P. M. & H. F. CHISAKI. 1957. Studies on *Amsinckia*. I. A synopsis of the genus, with a study of heterostyly in it. Amer. J. Bot. 44: 529–536.

RICHARDS, A. J. 1986. Plant Breeding Systems. Allen & Unwin, London.

SCHOU, O. & M. PHILIPP. 1984. An unusual heteromorphic incompatibility system: 3. On the genetic control of distyly and self-incompatibility in *Anchusa officinalis* L. (Boraginaceae). Theor. Appl. Genet. 68: 139–144.

URBAN, I. 1883. Monographie der Familie der Turneraceen. Jahrb. Bot. Gart. Berlin 2: 1–152.

VASUDEVAN NAIR, R. 1975. Heterostyly and breeding mechanism of *Nymphoides cristatum* (Roxb.) O. Kuntze. J. Bombay Nat. Hist. Soc. 72: 677–682.